AD_____

Award Number: W81XWH-13-1-0158

TITLE: Targeting the Immune System's Natural Response to Cell Death to Improve Therapeutic Response in Breast Cancers

PRINCIPAL INVESTIGATOR: Rebecca S. Cook

CONTRACTING ORGANIZATION: Vanderbilt University
NashvilleTN 37232

REPORT DATE: June 2014

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
June 2014	Annual Report	1 June 2013- 31 May 2014

4. TITLE AND SUBTITLE

Targeting the Immune System's Natural Response to Cell Death to Improve Therapeutic Response in Breast Cancers

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-13-1-0158

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Rebecca S. Cook

E-Mail: Rebecca.cook@vanderbilt.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Vanderbilt University
Nashville, TN, 37232

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Purpose: We have proposed experiments to test the hypothesis that MerTK-mediated efferocytosis by tumor associated macrophages (TAMs) is a major limitation to effective therapeutic responses, because efferocytosis of dying tumor cells drives production of wound-healing/Th2-like cytokines, limits anti-tumor immunity, and promotes tumor growth. **Scope:** Two Aims were proposed to test this hypothesis. The goal of **Aim 1** was to determine if MerTK-directed efferocytosis modulates cytokine expression, leukocyte infiltration, and growth of mouse mammary tumors, specifically testing the hypothesis that loss of MerTK would impair efferocytosis of dying tumor cells by TAMs, thus limiting production of Th2/WH cytokines in the tumor microenvironment (TME), resulting in decreased tumor growth and metastasis. The goal of **Aim 2** was to measure the impact of MerTK-directed efferocytosis on tumor re-emergence in therapeutically treated breast cancers, specifically testing the hypothesis that loss of MerTK-directed efferocytosis in the TME will limit Th2/WH cytokines, thereby preventing immune tolerance and tumor regrowth. **Major Findings and Up-to-Date Report of Progress:** As indicated in the Statement of Work, the major tasks for year 1 involve mouse breeding strategies to generate the genetic model with which we may test the role of efferocytosis in spontaneously arising mammary tumors. This has been accomplished according to schedule, such that we are now observing mice daily to assess tumor formation.

15. SUBJECT TERMS

Effercytosis, breast cancer, MerTK, therapeutic response, lapatinib, wound healing, cytokines, leukocytes, tumor associated macrophages.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	12	

Table of Contents

INTRODUCTION: We have proposed experiments to test the hypothesis that MerTK-mediated efferocytosis by tumor associated macrophages (TAMs) is a major limitation to effective therapeutic responses, because efferocytosis of dying tumor cells drives production of wound-healing/Th2-likecytokines, limits anti-tumor immunity, and promotes tumor growth. Two Aims were proposed to test this hypothesis. The goal of **Aim 1** was to determine if MerTK-directed efferocytosis modulates cytokine expression, leukocyte infiltration, and growth of mouse mammary tumors, specifically testing the hypothesis that loss of MerTK would impair efferocytosis of dying tumor cells by TAMs, thus limiting production of Th2/WH cytokines in the tumor microenvironment (TME), resulting in decreased tumor growth and metastasis. This Aim relies on the use of an immune competent mouse mammary tumor model with systemic loss of MerTK, measuring intra-tumoral leukocytes and tumor epithelial cell signaling using flow cytometry/CyTOF. This Aim also proposed using a novel cell co-culture model to assess MerTK-mediated efferocytosis by time-lapse microscopy. The goal of **Aim 2** was to measure the impact of MerTK-directed efferocytosis on tumor re-emergence in therapeutically treated breast cancers, specifically testing the hypothesis that loss of MerTK-directed efferocytosis in the TME will limit Th2/WH cytokines, thereby preventing immune tolerance and tumor regrowth. Like experiments proposed in Aim 1, those proposed in Aim 2 use an immune competent mouse mammary tumor model with systemic loss of MerTK or with pharmacological MerTK inhibition, measuring intra-tumoral leukocytes and tumor epithelial cell signaling in the post-therapeutic setting using flow cytometry/CyTOF, and monitoring post-treatment tumor progression.

BODY:

The Body of this Progress Report will discuss the tasks proposed for Year 1 (Quarters 1-4) in the approved Statement of Work, which include Tasks 1 and 2 (Please see Table 7 of the Statement of Work, which is shown below).

Table 7. Statement of Work Timeline

		Year	Year1				Year2				Year3			
		Quarter	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12
Aim 1	Task 1	Generate tumor-bearing mice												
	Task 2	Tumor formation &progression												
	Task 3	Tumor and tissue analysis												
	Task 4	Imaging of efferocytosis												
Aim 2	Task 5	Generate tumor-bearing mice												
	Task 6	Tumor formation												
	Task 7	Tumor treatment and growth												
	Task 8	Tumor and tissue analysis												

Task1. Generate tumor bearing mice.

Task 1 is completed, in accordance with the scheduled timeline. We used three rounds of breeding (as shown in Statement of Work Table 5, below) to generate 12 female MerTK$^{+/+}$NIC^{Cherry} mice and 12 female MerTK$^{-/-}$NIC^{Cherry} mice. These studies were completed in early Quarter 4 (March, 2014), resulting in a complete cohort (controls and experimentals) of age-matched siblings born between March 1, 2014 and March 20, 2014.

Table 5. Breeding Strategy to generate MerTK$^{+/+}$NIC^{Cherry} and MerTK$^{-/-}$NIC^{Cherry} female mice

step	# breeding pairs	Dam	Sire	Key Offspring Genotypes	Mendelian Frequency	# pups
1	8	MerTK$^{+/-}$	MMTV-NIC			screen 64
				MerTK$^{+/-}$ MMTV-NIC	♀ 25%	save 16 ♀ (for breeding
2	16	MerTK$^{+/-}$ MMTV-NIC	LSL-H2B-mCherry			screen 128
				MerTK$^{+/-}$ NIC^{Cherry}	♀ 12.5%	save 16 (for breeding)
				MerTK$^{+/-}$ NIC^{Cherry}	♂12.5%	save 6 (for breeding)
3	16	MerTK$^{+/-}$ NIC^{Cherry}	MerTK$^{+/-}$ NIC^{Cherry}			screen 128 pups
				MerTK$^{+/+}$ NIC^{Cherry}	♀ 9.375%	save 12 (N = 9 + contingency at 30%)
				MerTK$^{-/-}$ NIC^{Cherry}	♀ 9.375%	Save 12 (N = 9 + contingency at 30%)

Task 2. Tumor formation and progression.

Task 2 is ongoing, in accordance with the scheduled timeline. The average tumor latency for the MMTV-NIC mouse model is 6.5 months. Therefore, we anticipate that tumors in all mice will arise between late Quarter 5 and early Quarter 7. We have been palpating mouse mammary glands twice weekly to detect tumors since mice were 3 weeks of age. No tumors have been detected thus far. We will continue palpating mouse mammary glands to detect tumors until tumor formation is confirmed in at least 9 mice group, as indicated in Statement of Work.

KEY RESEARCH ACCOMPLISHMENTS:

- **Generated a cohort of mice that will form spontaneous mCherry+ tumors in efferocytosis-competent (MerTK$^{+/+}$) and efferocytosis-impaired (MerTK$^{-/-}$) backgrounds.**

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

- manuscripts, abstracts, presentations from this research: **none**
- licenses applied for and/or issued resulting from this research: **none**
- degrees obtained that are supported by this award: **none**
- development of cell lines, tissue or serum repositories: **Generated a cohort of mice that will form spontaneous mCherry+ tumors in efferocytosis-competent (MerTK$^{+/+}$) and efferocytosis-impaired (MerTK$^{-/-}$) backgrounds.**
- Infomatics: **none**
- funding applied for based on work supported by this award: **none**
- employment or research opportunities applied for and/or received based on experience/training supported by this award: **none**

CONCLUSION: The tasks required to test our hypothesis require a substantial breeding protocol that occupied the first year of the funding period. This was expected and outlined in the proposed Statement of Work. This burdensome breeding strategy is well worth the investment despite the delay in data acquisition, data analysis, publication, and tangible outcomes. This complex breeding strategy will produce the ideal model in which to test how macrophage-mediated efferocytosis in the tumor microenvironment affects tumor progression. This model is the only of its kind ever reported, and will have the advantages of a spontaneous tumor developing in its homotypic matrix in the context of a fully functional immune system. Once tumors develop, which is expected to occur in August 2014 through September 2014, analysis of this novel tumor model will begin, including flow cytometry/CyTOF to measure leukocyte populations, efferocytosis assays ex vivo, cytokine analyses, and therapeutic responses.

REFERENCES: None

APPENDICES: None

SUPPORTING DATA: None